



Dust, Endotoxin, Fungi, and Bacteria Exposure as Determined by Work Task, Season, and Type of Plant in a Flower Greenhouse

Trine Thilsing^{1,2*}, Anne Mette Madsen³, Ioannis Basinas⁴,
Vivi Schlünssen^{4,5}, Kira Tendal³ and Jesper Bælum^{1,2}

1. Research Unit for Occupational and Environmental Medicine, Institute of Clinical Research, University of Southern Denmark, Winsløvparken 19, 3., DK-5000 Odense C, Denmark

2. Research Unit of General Practice, Institute of Public Health, University of Southern Denmark, J.B Winsløvsvej 9A, DK-5000 Odense C, Denmark

3. The National Research Centre for the Working Environment, Lersø Parkallé 105, DK-2100 Copenhagen Ø, Denmark;

4. Department of Public Health, Section for Environment, Occupation and Health, Danish Ramazzini Centre, Aarhus University, Bartholins Allé 2, bg 1260, DK-8000 Aarhus C, Denmark

5. Department of Occupational Medicine, Danish Ramazzini Centre, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark

*Author to whom correspondence should be addressed. Tel: +45-65414990; fax: +45-65414988; e-mail: tthilsing@health.sdu.dk

Submitted 15 May 2014; revised 21 September 2014; revised version accepted 25 September 2014.

ABSTRACT

Background: Greenhouse workers are exposed to dust, endotoxin, fungi, and bacteria potentially causing airway inflammation as well as systemic symptoms. Knowledge about determinants of exposure is a prerequisite for efficient prevention through knowledge-based reduction in exposure. The objective of this study was to assess the occupational exposure in a flower greenhouse and to investigate the impact of work tasks on the intensity and variability in exposure.

Methods: Seventy-six personal full-shift exposure measurements were performed on 38 employees in a Danish flower greenhouse producing *Campanula*, *Lavandula*, Rhipsalideae, and *Helleborus*. The samples were gravimetrically analysed for inhalable dust. Endotoxin was assessed by the Limulus Amoebocyte Lysate test and culture-based quantification of bacteria and fungi was performed. Information on the performed tasks during sampling was extracted from the greenhouse electronic task logging system. Associations between log-transformed exposure outcomes, season, and work tasks were examined in linear mixed-effects regression with worker identity as random effect.

Results: Measured concentrations ranged between 0.04 and 2.41 mg m⁻³ for inhalable dust and between 0.84 and 1097 EU m⁻³ for endotoxin exposure, with the highest mean levels measured during *Lavandula* and *Campanula* handling, respectively. Personal exposure to fungi ranged between 1.8 × 10² and 3.4 × 10⁶ colony-forming units (CFU) m⁻³ and to bacteria between 1.6 × 10¹ and 4.2 × 10⁵ CFU m⁻³. Exposure to dust, endotoxin, fungi, and bacteria differed between seasons. Packing *Lavandula*, sticking, potting, and grading Rhipsalideae, and all examined tasks related to *Campanula* production except sticking increased dust exposure. Endotoxin exposure was increased during sticking *Campanula* and pinching or packing Rhipsalideae, and fungi exposure was elevated by subtasks performed in the research and development area for *Campanula*, and by potting, packing/dumping *Campanula*. Sticking and working with subtasks in the research and development area for *Campanula* increased bacteria exposure.

Conclusion: This study revealed moderate dust exposure levels compared to the levels observed in other greenhouse productions and other occupations with organic dust exposure such as farming. However, high exposures to bacteria and fungi were detected during selected tasks and the proposed health-based endotoxin exposure limit of 90 EU m⁻³ was exceeded in 30% of the samples, which may have health implications for the employees. Exposure levels were found to vary depending on the tasks performed, and thereby results can be used to direct task-based initiatives to reduce workplace exposures.

KEYWORDS: bacteria; exposure; dust; endotoxin; fungi; flower greenhouse; work task

INTRODUCTION

The intensive production of vegetables and flowers in greenhouses is performed with high indoor temperature and humidity, providing optimal growth conditions for a wide variety of fungi and bacteria. In addition to such natural dust components, the spread of biopesticides may contribute to the bioaerosol exposure in greenhouse settings (Hansen *et al.*, 2010a; Madsen, 2011; Madsen *et al.*, 2014b). Greenhouse workers are therefore potentially exposed to high levels of organic dust containing allergens, bacteria, fungi, and proinflammatory substances such as endotoxin (Madsen *et al.*, 2009; Hansen *et al.*, 2010b; Adhikari *et al.*, 2011; Hansen *et al.*, 2012). Numerous studies have shown organic dust exposure in occupational settings to cause airway inflammation as well as systemic symptoms (Davies *et al.*, 1988; Doekes *et al.*, 2004; Smit *et al.*, 2005; Smit *et al.*, 2006). Endotoxin, the most well investigated component of organic dust, is composed of lipopolysaccharides from the outer cell membrane of Gram-negative bacteria (Liebers *et al.*, 2008). Inhalation of endotoxin has been associated with health impairment including fever, headache, nose and throat irritation, chest tightness, cough, shortness of breath, wheezing, acute airway flow restriction, and inflammation (Schwartz *et al.*, 1994; Rylander, 2002; Wang *et al.*, 2003; Rylander, 2006). Exposure to fungi may be associated with the development of asthma, hypersensitivity pneumonitis, decline in lung function, and airway inflammation (Davies *et al.*, 1988; Farruggia and Bellia, 2001; Monsó *et al.*, 2002; Simon-Nobbe *et al.*, 2008) and exposure to allergens from plant pollen or biopesticides may give rise to IgE sensitization, allergic rhinitis and asthma (Groenewoud *et al.*, 2002a, b, c; Skousgaard *et al.*, 2010; Thilsing *et al.*, 2012). Studies on greenhouse workers have revealed lower ventilatory capacity compared to predicted values and higher prevalence of

rhinitis (Zuskin *et al.*, 1993), and respiratory and dermal symptoms compared to controls (Hanssen *et al.*, 2014).

Studies focusing on exposure have revealed personal dust exposures between 0.2 and 15 mg m⁻³ (median 1.6 mg m⁻³) and endotoxin exposures between 0.5 and 400 ng m⁻³ (median 32 ng m⁻³) among employees in cucumber and tomato greenhouses (Madsen *et al.*, 2009). The Danish occupational exposure limit (OEL) for total organic dust (3.0 mg dust m⁻³) was exceeded among 36 and 17% of the workers in the cucumber and tomato greenhouses, respectively, and the median endotoxin exposure (32 ng m⁻³~320 EU m⁻³) considerably exceeded the suggested health-based endotoxin exposure limit of 90 EU m⁻³ (DECOS, 2010; NEG, 2011). Only a limited number of studies have assessed the personal occupational bioaerosol exposure in flower greenhouses or the flower bulb trade (Monsó *et al.*, 2002; Radon *et al.*, 2002; Madsen, 2006; Spaan *et al.*, 2006; Smit *et al.*, 2008; Adhikari *et al.*, 2011). Only one of these included job titles for evaluation of endotoxin exposure (Smit *et al.*, 2008).

Knowledge about determinants of exposure in greenhouse settings is a prerequisite for efficient prevention through knowledge-based reduction in exposure. However, exposure measurements are often complicated by large exposure variability due to changes in tasks, activities, work processes, and locations over time (Burdorf, 2005). Valid registration of such work day changes therefore improves the possibility of evaluating determinants of exposure.

The objective of this study was to assess the occupational exposure to dust, endotoxin, fungi, and bacteria in a flower greenhouse and to investigate the impact of work tasks on the intensity and variability in exposure.

We used data from the greenhouse activity logging database to assess the individual minute-to-minute activity, and combined these data with personal exposure measurements.

METHODS

The study was performed in a Danish flower greenhouse over a 2.5-year period (June 2010–December 2012). The greenhouse produced *Campanula* (~14 mill. plants per year), *Lavandula* (*Lavandula stoechas*, ~1 mill. plants per year), *Rhipsalideae* (*Rhipsalidopsis* (Easter cactus) and *Schlumbergera* (Christmas cactus), ~3.5–4 mill. plants per year), and *Helleborus* (~50 000 plants per year) and had a total of 108 employees (Table 1). During high season for *Campanula* production (November–April), the number of employees rose to ~200. Thirty-eight permanently employed workers (22 women and 16 men) representing ~40% of the employees working inside the greenhouse participated in this study. The participants were recruited among employees at work on the sampling days selected. Sampling was performed on participants agreeing to carry the samplers during the entire working day.

Assessment of exposure

During the study period, 76 individual full-shift exposure measurements were carried out on 11 separate work days, covering all 4 seasons. Sampling dates were planned in collaboration with the greenhouse management in order to cover the most prevalent tasks during all four seasons. The number of exposure measurements distributed on dates are shown in Fig. 1. From each of the 38 participants, 1–6 (median: 2) exposure measurements were obtained.

Sampling of personal inhalable dust was performed using plastic GSP samplers (Gesamtstaubprobenahme, CIS by BGI, INC Waltham, MA, USA) with a flow of 3.5 l min⁻¹. This has been described elsewhere (Kenny *et al.*, 1997). Air flow on the pump was checked hourly with a rotameter and when necessary the flow was re-calibrated to ensure consistency. Sampling was commenced at work start with the intention of collecting dust during the entire working day. Each worker carried two samplers attached to the clothes near the breathing zone, one with a polycarbonate filter (pore size 1 µm, Osmonics Inc., Minnetonka, MN, USA) for culturable counts and one with a teflon filter (pore size 1 µm; Millipore, Bedford, MA, USA) for endotoxin and gravimetric analysis. Teflon filter was used

for these measures, as it has proven to be suitable for endotoxin extraction (Douwes *et al.*, 1995; Duquenne *et al.*, 2013), and as it has been used in several previous studies (Madsen, 2006). Each sampling day three teflon filters and one polycarbonate filter were brought as blank filters—i.e. were subjects to the same handling as the filters used in sampling, but no air was sampled through them. The three teflon filters were used for gravimetric analysis and one of them subsequently as blank filter for endotoxin analysis, whereas the polycarbonate filter served as a blank filter for quantification of microorganisms. Every afternoon, immediately after sampling all filters were carefully placed in a box and transported to the laboratory. Filters were stored over night at 22°C and 50% relative humidity.

Gravimetric analysis

The mass of the dust collected on the teflon filters was determined by weighing the filters before and after dust sampling (Sartorius Micro Balance, readability 0.001 mg, Sartorius AG, Goettingen, Germany). Before weighing, the filters were equilibrated at constant air temperature and humidity for 20–24 h (22°C and 50% rh ± 5%). The limit of detection when weighing the filters was between 0.012 and 0.0012 mg dust m⁻³. The detection limit was calculated as three times the standard deviation of 10 blanks and divided by the mean sampled volume. The data are presented as mg m⁻³ air.

Extraction of dust

The day after each sampling, the materials collected on filters were extracted. The dust on the teflon filters was extracted in 6.0 ml pyrogen-free water with 0.05% Tween 20 by orbital shaking (300 rpm) at room temperature for 60 min and centrifuging (1000 g) for 15 min. The supernatant was stored at -80°C until it was used for the endotoxin assay. In the morning on the day after sampling, the dust collected on polycarbonate filters was extracted by placing filters in 10 ml sterile solution (0.05% Tween 80, 0.85% NaCl) followed by orbital shaking for 15 min (500 rpm) at room temperature. Ten-fold dilution series were prepared and 100 µl aliquots were placed on agar plates for quantification of bacteria and fungi.

Quantification of endotoxin

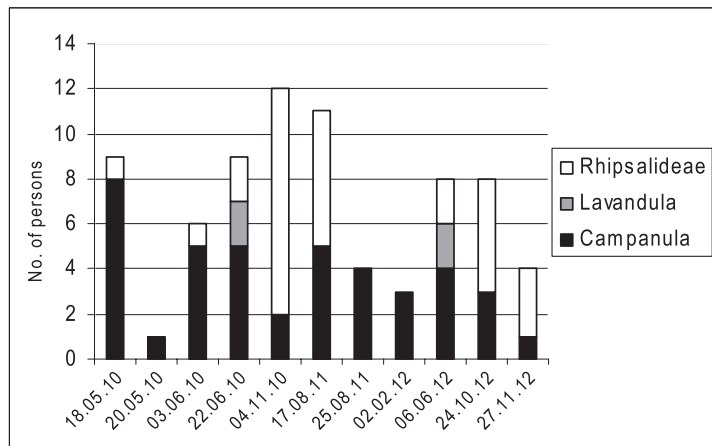
The supernatants from teflon filters were analysed (in duplicate) for endotoxin from Gram-negative bacteria using the kinetic Limulus

Table 1. Greenhouse and task characteristics

Greenhouse characteristics	
Size, m ²	200 000 m ² indoor greenhouse production, 60 000 m ² outdoor production
Production	<i>Campanula</i> (~14 mill. plants per year), <i>Lavandula</i> (~1 mill. plants per year), Rhipsalideae (~3.5–4 mill. plants per year), and <i>Helleborus</i> (~50 000 plants per year)
Ventilation	Natural (windows) and mechanical (fans and blowers)
Heating	Central heating
Task characteristics	
<i>Campanula</i>	
Research and development	Working in the Research and Development section. Main tasks performed: nursing, potting, cleaning sticking, retrieving cuttings
Sticking	Manually sticking cuttings into pots with peat
Potting	Transplanting the growing plants into larger pots
Nursing/spacing	Nursing plants/working by a conveyor, manually increasing the space between the pots to provide more space for each plant
Packing/dumping	Manually packing full grown potted plants individually in plastic covers/dumping substandard full grown plants into container
<i>Lavandula</i>	
Packing	Manually packing full grown potted plants individually in plastic covers
Rhipsalideae	
Research and development	Working in the Research and Development section. Main tasks performed: nursing, potting, cleaning, sticking, retrieving cuttings
Sticking/potting/grading	Manually sticking cuttings/transplanting the growing plants into larger pots/grading plants
Spacing	Working by a conveyor, manually increasing the space between pots to provide more space for each plant
Nursing/moving plants	Nursing plants/transferring tables with growing plants into other sections of the greenhouse
Pinching	Manually pinching the outer most branches of the plants to obtain the desired shape and induce maximum bud formation
Packing	Manually packing full grown potted plants individually in plastic covers

Amoebocyte Lysate test (Kinetic-QCL endotoxin kit, BioWhittaker, Walkersville, MA, USA) with β -glucan blocker. A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentrations in terms

of endotoxin units (EU) (10.0 EU $\approx$ 1.0 ng). The limit of detection was between 0.03 and 0.30 EU m⁻³, dependent on the sampling time. The data are presented as EU m⁻³ air. Endotoxin was below detection level on all blind filters.



1 Number of individual exposure measurements distributed on sampling date and type of plant.

Quantification of bacteria and fungi

The number of fungi in GSP polycarbonate samples culturable on Dichloran Glycerol agar (DG18 agar, Oxoid, Basingstoke, England) at 25°C were counted after 3 and 7 days of incubation. Due to different growth speeds, bacteria were quantified after 3 and 7 days of incubation on 100% Nutrient agar (Oxoid, Basingstoke, UK) with actidione [cycloheximide; 50 mg l⁻¹ (Serva, Germany)] at 25°C. The detection limit was between 7 and 61 colony-forming units (CFU) m⁻³ dependent on sampling time.

Work task records

The greenhouse used a task logging system where daily activities and their duration were registered electronically. Every worker used an electronic card to log in and out of the system each time a work task was commenced or ended. From the greenhouse database, individual's daily minute-to-minute registrations of work tasks (combination of work activity and plant) were retrieved. Data extracts were obtained for all days with individual exposure measurements. Initially, a total of 23 different task registrations on *Campanula*, *Rhipsalideae*, or *Lavandula* subspecies were represented in the dataset. There were no task registrations on *Helleborus*. Similar activity registrations, e.g. packing of different subspecies of *Campanula* or different genera of *Rhipsalideae* (*Schlumbergera* and *Rhipsalidopsis*) were grouped as 'Packing, *Campanula*' or 'Packing, *Rhipsalideae*'. In addition, six tasks with less than three registrations were merged with larger groups

according to plant type (*Campanula*, *Rhipsalideae*, or *Lavandula*), plant stage (cuttings, growing plants or fully grown plants), and similarity in activity. The resultant final dataset comprised a total of 12 different work tasks distributed on *Campanula* (research and development; sticking; potting; nursing/spacing; packing/dumping), *Rhipsalideae* (research and development; sticking/potting/grading; spacing; nursing/moving plants; pinching, packing), and *Lavandula* (Packing). A description of the included tasks is given in Table 1.

Statistical analysis

Exposure outcomes were log-transformed to obtain normally distributed data. Therefore, exposure distribution characteristics are summarized as both arithmetic mean (AM) and as geometrical mean (GM) with 95% confidence interval (95%CI), and geometric standard deviation (GSD). Mixed effect linear regression models were used to assess the relation between exposure levels and tasks. Tasks and season were included as fixed effects and worker as random effect to account for correlations between repeated measurements from the same worker (Peretz et al., 2002). Two analyses were performed. In the first analysis (model 1), tasks were included as continuous variables denoting the number of minutes spent on specific tasks for any given worker at any given date. In the second analysis (model 2), tasks were included as dichotomous variables (task performed yes/no). All 12 task variables were included in the modeling process that

involved backward stepwise selection until only task variables with a significance level <0.1 were retained. Season was included as a categorical variable with four levels, and a likelihood ratio test was performed to test the fit of the final models with and without season. A compound symmetry covariance structure was assumed, and estimations of variance components were based on the restricted maximum-likelihood method. Spearman correlation between the different exposure outcomes were calculated. Data were analyzed using the STATA statistical software, version 13 (StataCorp LP, TX, USA).

RESULTS

The exposure to dust, endotoxin, fungi, and bacteria by plant and season is shown in Table 2. Personal exposure to inhalable dust and endotoxin ranged between 0.04 and 2.41 mg m⁻³ (AM: 0.36 mg m⁻³, GM: 0.25 mg m⁻³, GSD: 1.10) and 0.84 and 1097 EU m⁻³ (AM: 96.9 EU m⁻³, GM: 44.4 EU m⁻³, GSD: 1.17), respectively. The endotoxin exposure was above 90 EU m⁻³ in 23 samples. Dust and endotoxin exposure differed significantly between plant types with higher dust and endotoxin exposure from *Campanula* than from *Rhipsalideae*, and with dust exposure from *Lavandula* significantly exceeding *Campanula* as well as *Rhipsalideae*. Endotoxin exposure from *Lavandula* was not significantly different from *Campanula* nor *Rhipsalideae*.

Personal exposure to fungi and bacteria ranged between 1.8×10^2 and 3.4×10^6 CFU m⁻³ (AM: 1.9×10^5 CFU m⁻³, GM: 3.1×10^4 CFU m⁻³, GSD: 1.24) and 1.6×10^1 and 4.2×10^5 CFU m⁻³ (AM: 3.3×10^4 CFU m⁻³, GM: 5.3×10^3 CFU m⁻³, GSD: 1.25), respectively. The fungi and bacteria exposure did not differ significantly between plants. Exposure to dust, endotoxin, fungi and bacteria differed significantly between seasons, with the lowest endotoxin exposure during fall and the highest bacteria exposure during winter. The correlations between dust and endotoxin ($r = 0.45$, $P = 0.0001$), and endotoxin and bacteria ($r = 0.41$, $P = 0.0004$) exposure were moderate. No significant correlation between dust and fungi ($r = 0.10$, $P = 0.38$), dust and bacteria ($r = 0.12$, $P = 0.32$), fungi and endotoxin ($r = -0.15$, $P = 0.19$) or fungi and bacteria ($r = -0.22$, $P = 0.08$) was found.

The daily sampling time ranged between 47 and 470 min (median 348 min, mean 324 min). Two

samples covered only part of the working day due to technical problems or discomfort from carrying the samplers. The mean daily sampling time did not differ between type of plant or seasons. Only one participant worked with different plant types on the same working day, whereas 9 out of the 21 participants with more than one full-shift exposure measurement worked with different plant types on different days.

Table 3 shows the mean (AM and GM) exposure measured on days with single task performance—i.e. days with only on task registered per participant ($n = 69$). The highest mean dust exposure (AM: 0.74 mg m⁻³, GM: 0.53 mg m⁻³) was observed from packing *Lavandula* and the highest endotoxin (AM: 114.1 EU m⁻³, GM: 105.5 EU m⁻³) and bacteria (AM: 1.7×10^5 CFU m⁻³, GM: 1.0×10^5 CFU m⁻³) exposure arose from sticking *Campanula*. Packing/dumping *Campanula* led to the highest mean fungi (AM: 5.2×10^5 CFU m⁻³, GM: 1.3×10^5 CFU m⁻³) exposure. Working in the research and development area was represented by one single task measurement only and is therefore not considered representative.

Table 4 shows the results from model 1 with tasks (continuous variables) and season included as fixed effects. The final models comprised of six tasks for dust, four for endotoxin, three for fungi, and three for bacteria. In total, 10 of the 12 tasks were included in one or more of the final models. Dust and bacteria exposure was increased during work in the research and development area for *Campanula*. One hour work in this area increased exposure to dust and bacteria by 121 and 147%, respectively. The average time spent in the research and development area was 89 min (median 100 min). In comparison, the average time spent sticking *Campanula* was 265 min. (median 322 min), increasing the endotoxin and bacteria exposure by 26 and 52% per hour, respectively. Potting and packing/dumping *Campanula* were other time consuming tasks (average work time 281 and 291 min, respectively) increasing exposure to dust by 20 and 12%, and to fungi by 26 and 48% per hour. Among the tasks on *Rhipsalideae* sticking/potting/grading increased dust exposure (12% per hour), and packing *Lavandula* also significantly increased dust exposure (42% per hour). The statistical models explained 28, 40, 42, and 51% of the total variation in dust, endotoxin, fungi, and bacteria exposure, respectively. The majority of the total variation was constituted by the within worker

Table 2. Exposure to dust (mg m^{-3}), endotoxin (EU m^{-3}), fungi (CFU m^{-3}), and bacteria (CFU m^{-3}) among Danish greenhouse workers depending on type of plant and season

N	Sampling time per day, median, (range)	Dust ^{a,*,b,*}			Endotoxin ^{a,*,b,*}			Fungi ^{b,***}			Bacteria ^{b,***}						
		n	AM	GM (GSD)	95%CI	n	AM	GM (GSD)	95%CI	n	AM	GM (GSD)	95%CI				
All measurements																	
38	348 (47–470)	75	0.36	0.25 (1.10)	0.21–0.30	76	96.9	44.4 (1.17)	32.4–60.8	76	1.9 × 10 ⁵	3.1 × 10 ⁴	2.1 × 10 ⁴ –4.8 × 10 ⁴	69	3.3 × 10 ⁴	5.3 × 10 ³ (1.25)	3.4 × 10 ³ –8.2 × 10 ³
Among persons working with																	
<i>Campanula</i>																	
30	335 (47–460)	40	0.43	0.31 ^a (1.14)	0.24–0.40	41	123.7	65.6 ^a (1.20)	45.7–94.4	41	3.2 × 10 ⁵	4.0 × 10 ⁴	2.0 × 10 ⁴ –8.0 × 10 ⁴	37	5.2 × 10 ⁴	7.5 × 10 ³ (1.40)	3.9 × 10 ³ –1.5 × 10 ⁴
<i>Lavandula</i>																	
4	217 (110–352)	4	0.74	0.53 ^b (2.35)	0.10–2.84	4	50.4	40.0 ^{a,b} (2.01)	10.2–156.5	4	5.5 × 10 ⁴	3.6 × 10 ⁴ (2.54)	5.7 × 10 ³ –2.2 × 10 ⁵	3	6.9 × 10 ³	5.2 × 10 ³ (3.67)	4.1 × 10 ² –6.7 × 10 ⁴
Rhipsalideae																	
15	330 (60–470)	30	0.22	0.18 ^c (1.13)	0.14–0.22	30	69.0	27.1 ^b (1.35)	15.0–48.9	30	3.4 × 10 ⁴	2.2 × 10 ⁴ (1.26)	1.4 × 10 ⁴ –3.4 × 10 ⁴	28	1.1 × 10 ⁴	3.5 × 10 ³ (1.37)	1.9 × 10 ³ –6.5 × 10 ³
During																	
Spring																	
8	310 (85–375)	10	0.44	0.38 ^a (1.21)	0.26–0.56	10	190.9	96.67 (1.47)	45.3–206.5	10	4.5 × 10 ⁵	1.1 × 10 ⁵ (2.04)	2.8 × 10 ⁴ –4.6 × 10 ⁵	10	4.3 × 10 ³	3.5 × 10 ³ (1.27)	2.2 × 10 ³ –5.6 × 10 ³
Summer																	
29	334 (60–460)	37	0.44	0.28 ^a (1.17)	0.21–0.38	38	98.9	53.9 (1.21)	37.3–78.0	38	1.9 × 10 ⁵	2.7 × 10 ⁴ (1.35)	1.5 × 10 ⁴ –4.9 × 10 ⁴	31	2.9 × 10 ⁴	4.5 × 10 ³ (1.40)	2.3 × 10 ³ –8.8 × 10 ³
Fall																	
14	348 (125–470)	25	0.21	0.17 ^b (1.14)	0.14–0.22	25	50.4	21.2 ^a (1.40)	11.0–40.7	25	1.1 × 10 ⁵	3.5 × 10 ⁴ (1.36)	1.9 × 10 ⁴ –6.3 × 10 ⁴	25	1.5 × 10 ⁴	4.6 × 10 ³ (1.44)	2.3 × 10 ³ –9.5 × 10 ³
Winter																	
3	301 (47–371)	3	0.35	0.33 ^a (1.70)	0.12–0.93	3	144.8	134.2 (1.85)	40.2–447.9	3	1.3 × 10 ⁵	1.1 × 10 ⁵ (1.95)	3.1 × 10 ³ –4.2 × 10 ⁵	3	3.1 × 10 ⁵	3.0 × 10 ³ (1.57)	1.2 × 10 ³ –7.2 × 10 ³

AM, arithmetic mean; GM, geometrical mean; GSD, geometric standard deviation; 95%CI, 95% confidence interval for GM; N, number of workers, n, number of measurements.

^aSignificant difference between plants.

^bSignificant difference between season.

* $P < 0.05$, ** $P < 0.01$. Plants or seasons with different signs (α , β , δ) designate significant ($P < 0.05$) difference.

Table 3. Personal exposure to dust (mg m^{-3}), endotoxin (EU m^{-3}), fungi (CFU m^{-3}), and bacteria (CFU m^{-3}) from days with single task performance in a Danish flower greenhouse

		<i>n</i>	Dust			Endotoxin			Fungi			Bacteria		
			AM	GM	GSD	AM	GM	GSD	AM	GM	GSD	AM	GM	GSD
Flower	Task													
<i>Campanula</i>														
	Research and development	1	0.24	0.24	nm	98.6	98.6	nm	8.3×10^5	8.3×10^5	nm	3.1×10^3	3.1×10^3	nm
	Sticking	8	0.21	0.16	1.39	114.1	105.5	1.20	4.7×10^3	2.5×10^3	1.62	1.7×10^5	1.0×10^5	1.66
	Potting	6	0.44	0.38	1.34	112.0	88.8	1.46	4.6×10^5	8.4×10^4	2.94	6.4×10^3	5.0×10^3	1.54
	Nursing/ spacing	3	0.34	0.33	1.30	48.0	47.9	1.12	3.5×10^4	1.8×10^4	6.07	2.3×10^3	9.8×10^2	8.51
	Packing/ dumping	18	0.36	0.28	1.21	106.5	36.6	1.40	5.2×10^5	1.3×10^5	1.58	3.4×10^3	2.3×10^3	1.31
<i>Lavandula</i>														
	Packing	4	0.74	0.53	2.35	50.4	40.0	2.01	505×10^4	3.6×10^4	2.54	6.9×10^3	5.2×10^3	3.67
Rhipsalideae														
	Research and development	4	0.18	0.18	1.24	25.7	12.1	3.81	4.2×10^4	1.2×10^4	10.01	4.0×10^3	2.9×10^3	2.09
	Sticking/ potting/ grading	5	0.44	0.35	1.64	44.4	34.6	1.67	1.7×10^4	1.2×10^4	1.80	3.6×10^3	3.2×10^3	1.42
	Spacing	3	0.12	0.11	1.54	35.3	17.3	10.65	3.7×10^4	3.5×10^4	1.59	1.4×10^4	1.4×10^4	2.40
	Nursing/ moving plants	2	0.06	0.06	3.03	1.24	1.2	8.72	2.2×10^4	1.9×10^4	34.20	1.4×10^3	2.1×10^2	1.9×10^7
	Pinching	6	0.13	0.13	1.20	156.6	77.6	2.11	1.9×10^4	1.7×10^4	1.32	4.7×10^3	2.6×10^3	2.44
	Packing	9	0.24	0.21	1.20	46.4	30.1	1.55	5.5×10^4	4.6×10^4	1.27	2.3×10^4	7.0×10^3	1.79

AM, arithmetic mean; GM, geometric mean; GSD, geometric standard deviation, nm, not measured; *n*, number of measurements with active task performance.

variability, and the final statistical models explained 0, 35, 37, and 51% of this variation in dust, endotoxin, fungi and bacteria exposure, respectively. In comparison, 99.7% or more of the between worker variability for dust, endotoxin and fungi were explained by the model. The explained between worker variability for bacteria exposure was reported as 0%, as the naïve variance estimate as well as the modeled estimate was close to zero.

Table 5 shows results from model 2 with tasks included as dichotomous variables. The final models included six tasks for dust, three for endotoxin, four for fungi, and four for bacteria, explaining 33, 39, 42, and 53% of the total variation in exposure, respectively.

As for the modeling on continuous data, the total variation was largely constituted by the within worker variability, and the final statistical model explained between 9 and 53% of this variation.

Packing *Lavandula*, sticking, potting, and grading Rhipsalideae, and all tasks related to *Campanula* production except sticking increased dust exposure. The tasks with the largest effect on dust exposure was packing *Lavandula*, potting *Campanula*, and working in the research and development area. Fungi exposure was increased by potting, packing/dumping *Campanula*, and by working in the research and development area for *Campanula*. Endotoxin and bacteria exposure was increased by sticking *Campanula*, and endotoxin

Table 4. Effect of work tasks modelled as continuous variables (time spent in min) on the log-transformed level of exposure to dust (mg m⁻³), endotoxin (EU m⁻³), fungi (CFU m⁻³), and bacteria (CFU m⁻³) among Danish greenhouse workers.

	<i>n</i>	MDN minutes, (range)	Dust			Endotoxin			Fungi			Bacteria		
			β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>
Intercept			-1.67	0.28	<0.0001	4.50	0.34	<0.0001	10.60	0.53	<0.0001	7.92	0.41	<0.0001
Season														
	10	310 (85–375)	Ref			Ref			Ref			Ref		
Spring														
Summer	38	334 (60–460)	-0.20	0.26	0.44	-0.66	0.38	0.084	-0.91	0.52	0.081	0.27	0.47	0.954
Fall	25	348 (125–470)	-0.20	0.29	0.50	-1.46	0.42	<0.0001	-0.33	0.57	0.564	0.50	0.49	0.310
Winter	3	301 (47–371)	0.55	0.48	0.25	-0.54	0.75	0.47	-2.75	1.01	0.006	3.02	0.92	0.001
Flower														
Task														
Campanula														
	4	100 (37–118)	0.0132 (2.21)	0.0040	0.001							0.0151 (2.47)	0.0076	0.047
Research and development														
Sticking	13	322 (47–402)				0.0039 (1.26)	0.0012	0.001	-0.0034 (0.82)	0.0016	0.036	0.0070 (1.52)	0.0016	<0.0001
Potting	8	231 (137–460)	0.0031 (1.20)	0.001	0.002				0.0038 (1.26)	0.0019	0.044			
Nursing/spacing	3	376 (333–380)	0.0021 (1.13)	0.0012	0.076									
Packing/dumping	19	292 (139–369)	0.0019 (1.12)	0.0007	0.008				0.0065 (1.48)	0.0013	<0.0001			
Lavandula														
	4	218 (110–352)	0.0058 (1.42)	0.0016	<0.0001									
Packing														
Rhipsalideae														
	4	335 (60–435)				-0.0038 (0.80)	0.0017	0.026						
Research and development														
Sticking/potting/grading	5	361 (271–464)	0.0019 (1.12)	0.0009	0.034									
Spacing	3	365 (346–413)												
Nursing/moving plants	3	348 (10–437)				-0.0073 (0.65)	0.0021	<0.0001				-0.0069 (0.66)	0.0024	0.004
Pinching	7	389 (134–470)				0.0023 (1.15)	0.0011	0.048						
Packing	10	249 (139–469)												

Table 4. Continued

	<i>n</i>	MDN minutes, (range)	Dust			Endotoxin			Fungi			Bacteria		
			β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>
σ^2 (naïve estimate)			0.0006 (0.18)			0.00 (0.04)			0.00 (0.19)			0.00 (0.00)		
σ^2 (naïve estimate)			0.47 (0.47)			1.14 (1.86)			1.95 (3.18)			1.63 (3.33)		
Explained variability														
Between worker			99.7%			100%			100%			0%		
Within worker			0%			35%			37%			51%		
Total			28%			40%			42%			51%		

CF, factor change in exposure for 60 min of task performance ($= e^{(\beta \times 60)}$); MDN, median time spent on a task estimated only for positive responses; *n*, number of measurements; β , regression coefficient; *e*, standard error; *P*, *P* value; σ^2 , between-worker variance; σ^2 , within-worker (day-to-day) variance.

exposure also by pinching Rhipsalideae. Sticking *Campanula* was the task with the largest influence on bacteria as well as endotoxin exposure.

When comparing the number and direction of the effect on exposure for the tasks included in models 1 and 2 it is clear, that the final models for dust included the exact same task variables. The final models for fungi were also very similar with only one additional task included in model 2. For endotoxin and bacteria exposure, the models differed some more with respect to the number and selection of task variables included. For endotoxin, one task was included in model 2 but not in model 1, and two tasks were included in model 1 but not in model 2. For bacteria, the corresponding numbers were two and one. However, in general, among tasks included in both models no opposing direction of effect on exposure was seen between the models.

DISCUSSION

To our knowledge, this is the first study to assess work task determinants of exposure among workers in a flower greenhouse. This study shows the individual fungi and bacteria exposure to be influenced by season, and the exposure to dust and endotoxin to be influenced by type of plant as well as season. In addition, the performed tasks significantly influence exposure, with a general difference between tasks related to high exposure to dust and fungi on one side, and bacteria and endotoxin on the other. The final statistical models including tasks and season as fixed effects explain up to 53% of the total variation in exposure.

Data were sampled during a 2.5-year period, and measurements were performed on normal working days covering all four seasons. Analyses were based on individual electronically registered minute-to-minute task performance in conjunction with personal full shift exposure measurements. Compared to traditionally used self-administered activity diaries the data quality of this study is high with less potential recall and information bias. Furthermore, the tasks included in the models are tasks commonly performed in this type of flower greenhouse and the data therefore provide a solid basis for assessing important task determinants of exposure. The derived estimates may, however, be affected by the limited number of observations on some of the tasks, and by the fact that the tasks were defined by the greenhouse

Table 5. Effect of work tasks modelled as dichotomous variables on the log-transformed level of exposure to dust (mg m^{-3}), endotoxin (EU m^{-3}), fungi (CFU m^{-3}), and bacteria (CFU m^{-3}) in a Danish flower greenhouse

	<i>n</i>	Dust			Endotoxin			Fungi			Bacteria		
		β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>	β (CF)	<i>e</i>	<i>P</i>
Intercept		-2.06	0.32	<0.0001	4.40	0.34	<0.0001	10.42	0.61	<0.0001	8.15	0.42	<0.0001
Season													
	10	Ref.			Ref.			Ref.			Ref.		
Spring	38	0.039	0.27	0.885	-0.83	0.39	0.031	-0.76	0.58	0.192	0.25	0.46	0.581
Summer	25	0.15	0.32	0.632	-2.39	0.46	<0.0001	-0.05	0.66	0.944	0.42	0.48	0.384
Fall	3	0.94	0.49	0.058	-1.21	0.79	0.124	-1.55	1.17	0.19	2.15	0.92	0.019
Winter													
Task													
Flower													
<i>Campanula</i>													
	4	1.71 (5.53)	0.38	<0.0001				1.95 (7.03)	0.85	0.022			
Research and development													
Sticking	13				1.71 (5.53)	0.38	<0.0001	-1.85 (0.16)	0.55	0.001	2.32 (10.18)	0.47	<0.0001
Potting	8	1.22 (3.39)	0.33	<0.0001				1.33 (3.78)	0.67	0.048			
Nursing/spacing	3	0.92 (2.51)	0.42	0.03									
Packing/dumping	19	0.70 (2.01)	0.22	0.001				1.81 (6.11)	0.41	<0.0001	-1.51 (0.22)	0.77	0.051
											-0.72 (0.49)	0.38	0.06
<i>Lavendula</i>													
	4	1.37 (3.94)	0.38	<0.0001									
Packing													
Rhipsalideae													
	4												
Research and development													
Sticking/potting/grading	5	0.95 (2.56)	0.34	0.005									
Spacing	3												
Nursing/moving plants	3												
Pinching	7				1.86 (6.42)	0.46	<0.0001						
Packing	10				1.35 (3.86)	0.47	0.004				-2.38 (0.09)	0.77	0.002

Table 5. Continued

<i>n</i>	Dust			Endotoxin			Fungi			Bacteria		
	β (CF)	ϵ	<i>P</i>	β (CF)	ϵ	<i>P</i>	β (CF)	ϵ	<i>P</i>	β (CF)	ϵ	<i>P</i>
σ^2 (naïve estimate)	0.007 (0.18)			0.00 (0.04)			0.00 (0.19)			0.00 (0.00)		
σ^2 (naïve estimate)	0.43 (0.47)			1.16 (1.86)			1.97 (3.18)			1.55 (3.33)		
Explained variability												
Between worker	96%			100%			100%			0%		
Within worker	9%			38%			38%			53%		
Total	33%			39%			42%			53%		

CF, factor change in exposure if task is performed ($= \epsilon^2$); ϵ = standard error; *P* = *P* value; σ^2_{bet} = between-worker variance; σ^2_{w} = within-worker (day-to-day) variance; *n*, number of measurements; β , regression coefficient.

for administrative purposes and not for research on occupational hygiene. The sampling strategy aimed at obtaining representative samples and the number of samples collected during plant-specific tasks reflected the relative annual production share from each plant type. Still the relatively low number of sampling days may impose risk of chance observations. Furthermore, only one greenhouse was included in the analyses limiting the generalisation of the results to other greenhouse productions.

Data were analysed with tasks modelled as continuous (model 1) as well as dichotomous (model 2) variables. In principle, model 2 indicates whether performance of a specific task is important per se, and model 1 indicates whether duration of the task is important in determining the levels of exposure. Short durational tasks may not contribute significantly to an 8 h time weighted average concentration and therefore do not turn up as explanatory variables in models based on dichotomous task variables. On the other hand, tasks with limited time variation between workers may not turn up as explanatory variables in models based solely on task duration (Kromhout *et al.*, 1994). Therefore, it is not surprising that the resulting final models by the two approaches differed slightly with regard to the number and selection of tasks included. However, the direction of effects were similar in both models for all four outcome variables. The explained variabilities (between and within worker and total variability) of the two models were also comparable. Most (98–100%) of the variability in exposure to dust, endotoxin, fungi, and bacteria was allocated to the temporal within-worker component. This is in accordance with recent findings on dust and endotoxin exposure in livestock farming (Basinas *et al.*, 2012b; Basinas *et al.*, 2013). The final statistical models explained between 28 and 53% of the total variation, which is comparable to studies assessing determinants of organic dust exposure in agricultural settings (Preller *et al.*, 1995; Samadi *et al.*, 2012; Basinas *et al.*, 2013; Basinas *et al.*, 2014). Results of the mixed effect analyses in this study correspond well with the task-based exposure levels measured during single task performance (Table 3). The large variation in measured exposure for some of the tasks may, however, influence the accuracy of the corresponding estimates derived.

Our analyses indicated potting and packing/dumping *Campanula* as well as working in the research and

development area for *Campanula* to be important determinants of dust and fungi exposure. Packing *Campanula* is one of the major tasks covering a significant part of the total work load in the greenhouse. The relatively vigorous handling during packing/dumping of the flowers may increase the personal exposure. In addition, the age and size of the plants may be important, as previous studies on greenhouse tomato plants have shown higher exposure to dust and fungi from working with mature or senescent plants than from working with premature plants (Hansen *et al.*, 2012). Work in the research and development area is less time consuming than packing, and in the present dataset the task was represented by four measurements only. Therefore, the size of the estimates related to this task has to be interpreted with caution. The research and development task was performed in a separate building and covered some of the same (sub)tasks as in the primary production (e.g. sticking, nursing, potting etc.), but the duration of each (sub)task was not specified in detail in the greenhouse database. Our own records of (sub)task performance during the four registrations included tasks related to potting and cleaning, which corresponds well with the increased dust and fungi exposure during potting *Campanula* in the primary production. Other tasks which significantly increased dust exposure included packing *Lavandula*, nursing or spacing *Campanula* and sticking, potting, or grading Rhipsalideae.

Personal endotoxin exposure was increased during sticking *Campanula* and pinching Rhipsalideae. A study among workers in the flower bulb trade showed large variation in endotoxin exposure depending on job title with highest exposure among operators, packers, and crop growers (Spaan *et al.*, 2006). However, the tasks performed here are difficult to compare to the tasks performed in the flower bulb trade. Another study has shown significant endotoxin release during handling of peat (Airaksinen *et al.*, 2005). Therefore, a machine filling peat in pots located at the far end of the sticking area most likely contributes to the overall endotoxin load in this study, although this has not been confirmed by measurements.

Sticking *Campanula* was associated with increased exposure to bacteria. In the sticking area, spraying with biopesticides containing the Gram-positive bacteria *Bacillus thuringiensis israelensis* (*Bti*) is performed before sticking. Spraying is automatically performed

using a spraying boom. Afternoon measurements from stationary and personal samplers in the sticking area have revealed mean *Bti* exposure levels of 1.7×10^5 and 4.0×10^5 CFU m⁻³, respectively (Madsen *et al.*, 2014b). The corresponding total bacteria load was 4.6×10^5 and 5.5×10^5 CFU m⁻³, respectively, suggesting that the spraying of *Bti* before sticking is an important source of bacteria exposure (Madsen *et al.*, 2014b).

Although the electronic-based task registrations of this study provided a solid basis for dose-response estimations, the fact that not all subtasks were coded separately (e.g. spreading of biopesticides and (sub)tasks related to research and development activities) complicates the interpretation of the results. However, it seems that working with the young *Campanula* plants (e.g. sticking) generally increases endotoxin and bacteria exposure, whereas working with the full grown plants (e.g. packing/dumping) mainly increases exposure to dust and fungi. As task determinants of exposure have never previously been assessed in flower greenhouses, we are not able to relate our findings to other similar studies.

The dust exposure levels measured in this study exceeded previously reported personal exposure levels among flower and ornamental plant growers producing *Gerbera* spp, *Dianthus* spp, *Gladiolus* spp, *Chrysanthemum* spp, or *Geranium* spp (Monsó *et al.*, 2002; Radon *et al.*, 2002), were similar to the levels found in cut flower nurseries producing tulips and roses, and were lower than the levels reported from the flower bulb trade (Spaan *et al.*, 2006). The dust exposure was moderate compared to exposures reported from tomato and cucumber greenhouses (Madsen *et al.*, 2009; Hansen *et al.*, 2012; Madsen *et al.*, 2014a) and similar to or lower than exposures measured in mushroom, cucumber, paprika, and chicory nurseries (Spaan *et al.*, 2006), and in open fields growing cabbage, broccoli, and celeriac (Hansen *et al.*, 2012). None of the measurements exceeded the Danish OEL of 3 mg total dust m⁻³ (Arbejdstilsynet, 2007) corresponding to ~4.7 mg inhalable dust m⁻³ (Madsen *et al.*, 2009).

When comparing endotoxin results between different studies one should always keep in mind, that differences in sampling and analytical protocols may affect the outcome (Duquenne *et al.*, 2013). Compared to the endotoxin levels measured in other greenhouse

settings it seems, however, that the endotoxin exposure was higher than or similar to the levels found among flower and ornamental plant growers (Monsó *et al.*, 2002; Radon *et al.*, 2002; Spaan *et al.*, 2006), but lower than the level measured in the flower bulb trade (Spaan *et al.*, 2006; Smit *et al.*, 2008). Compared to the mean level previously reported from Danish cucumber and tomato greenhouses (AM: 67 ng m^{-3} $\sim 670 \text{ EU m}^{-3}$) (Madsen *et al.*, 2009) and from Dutch mushroom, chicory, tomato, cucumber, and paprika nurseries (GM: $69\text{--}160 \text{ EU m}^{-3}$) (Spaan *et al.*, 2006), the present endotoxin level was low, and considerably lower than the level measured during selected tasks such as clearing of tomato (Madsen *et al.*, 2009) and cucumber plants (Madsen *et al.*, 2009; Madsen *et al.*, 2014a). However, the mean endotoxin exposure was higher than the level measured during many other common tasks in tomato greenhouses, e.g. walking up and down the aisles, nipping leaves (Madsen *et al.*, 2014a) and harvesting tomatoes (Madsen *et al.*, 2009; Madsen *et al.*, 2014a). Although there is no officially acknowledged OEL for endotoxin the Nordic Expert Group and the Dutch National Health Council recently proposed a health-based endotoxin exposure limit of 90 EU m^{-3} (DECOS, 2010; NEG, 2011). In this study 30% of the endotoxin measurements were above this limit.

The personal exposure levels of fungi and bacteria were lower (Radon *et al.*, 2002) or higher (Monsó *et al.*, 2002) than the levels previously reported among workers in flower greenhouses. Compared to the exposure levels reported from Danish cucumber and tomato greenhouses the fungi levels were similar to (Madsen *et al.*, 2014a) or lower (Hansen *et al.*, 2012; Madsen *et al.*, 2014a), and the bacteria levels measured were above as well as below (Madsen *et al.*, 2014a). No officially acknowledged threshold limits are available for fungi and bacteria, but a lowest observed effect level (LOEL) of $\sim 10^5 \text{ spores m}^{-3}$ for diverse fungal species have been suggested for non-sensitized populations (Eduard, 2009).

Culture-based measures of bacteria and fungal exposure may result in lower exposure estimates than other available assessment methods (Eduard and Heederik, 1998). Therefore, the suggested LOEL of $10^5 \text{ fungal spores m}^{-3}$ (Eduard, 2009) may have been extensively exceeded on some of the participants of this study. Occupational exposure to fungi (Davies

et al., 1988; Farruggia and Bellia, 2001; Monsó *et al.*, 2002; Simon-Nobbe *et al.*, 2008) and bacteria (Lacey and Dutkiewicz, 1994) may cause respiratory symptoms. It is unknown whether the exposures measured here have caused such health problems, but the very high levels measured during some of the tasks could pose a problem. Previous studies on occupational exposure to *Bacillus thuringiensis israelensis* (Bti) revealed that regular use of Bti may increase the risk of IgE sensitization, but no direct relation with respiratory symptoms was found (Bernstein *et al.*, 1999; Doekes *et al.*, 2004; Baelum *et al.*, 2012). The dust exposure level was low in most samples in this study, whereas the concentration of endotoxin exceeded the proposed exposure limit of 90 EU m^{-3} (DECOS, 2010; NEG, 2011). As endotoxin is a very potent inducer of inflammation the exposure levels measured may represent an occupational risk to the employees, and result in symptoms like cough, shortness of breath, wheezing (Wang *et al.*, 2003; Latza *et al.*, 2004; Smit *et al.*, 2008), chronic bronchitis (Basinas *et al.*, 2012a), and decreased lung function (Portengen *et al.*, 2005). Our findings that the level of endotoxin exposure increases during sticking *Campanula* and pinching Rhipsalideae suggest that focus on preventive measures during these particular tasks would be relevant to reduce exposure. In addition, the high fungi exposure from potting and packing/dumping *Campanula* and the high bacteria exposure during sticking *Campanula* may call for preventive measures. Recently, the bti exposure was reduced from 3.0×10^5 to $1.8 \times 10^4 \text{ CFU m}^{-3}$ by placing a cover around the spray boom applying the biopesticide (Madsen *et al.*, 2014b). In addition, changes in task performance, job rotation, personal protective equipment, or increased ventilation could be relevant initiatives, but further studies are needed to test different preventive strategies.

CONCLUSIONS

In conclusion, this study is the first to describe how the occupational exposure to dust, endotoxin, fungi, and bacteria in a flower greenhouse varies depending on flower, season, and the tasks performed. The dust exposure levels measured were moderate compared to the levels observed in other greenhouse productions and other occupations with organic dust exposure such as farming. However, the concentration

of fungi, bacteria, and endotoxin were high in many of the samples, which may have health implications for the employees. In general, the majority of tasks related to *Campanula* production increased exposure to one or more of the dust components, where as only few tasks related to Rhipsalideae production increased exposure. Exposure levels varied significantly between tasks, and high levels of endotoxin exposure were measured during sticking *Campanula* and pinching Rhipsalideae. Exposure to fungi was increased during packing/dumping and potting *Campanula*, and exposure to bacteria was particularly high during sticking *Campanula*, most likely due to the spread of bacteria-based biopesticides in that working area. Results of this study therefore indicate which specific tasks to focus on when implementing preventive measures to reduce exposure.

FUNDING

The Danish Working Environment Research Fund (20090066435/4 and 20100020195/3); National Research Centre for the Working Environment.

ACKNOWLEDGEMENTS

We highly appreciate the help we have received from the management and employees of the participating greenhouse. A special thanks to the production advisor and secretary for invaluable help with extraction of data from the greenhouse database. Also thanks to Margit Wagtberg Frederiksen who has done all the laboratory analyses. All authors declare no conflict of interest related to this manuscript.

REFERENCES

- Adhikari A, Gupta J, Wilkins JR 3rd *et al.* (2011) Airborne microorganisms, endotoxin, and (1→3)-β-D-glucan exposure in greenhouses and assessment of respiratory symptoms among workers. *Ann Occup Hyg*; 55: 272–85.
- Airaksinen S, Heiskanen ML, Heinonen-Tanski H *et al.* (2005) Variety in dustiness and hygiene quality of peat bedding. *Ann Agric Environ Med*; 12: 53–9.
- Arbejdstilsynet. (2007) At-vejledning. Grænseværdier for stoffer og materialer (The Danish Working Authority. Work place exposure limits). pp 1–84.
- Baelum J, Larsen P, Doekes G *et al.* (2012) Health effects of selected microbiological control agents. A 3-year follow-up study. *Ann Agric Environ Med*; 19: 631–6.
- Basinas I, Schlünssen V, Heederik D *et al.* (2012a) Sensitisation to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis. *Occup Environ Med*; 69: 99–106.
- Basinas I, Schlünssen V, Takai H *et al.* (2013) Exposure to inhalable dust and endotoxin among Danish pig farmers affected by work tasks and stable characteristics. *Ann Occup Hyg*; 57: 1005–19.
- Basinas I, Sigsgaard T, Erlandsen M *et al.* (2014) Exposure-affecting factors of dairy farmers' exposure to inhalable dust and endotoxin. *Ann Occup Hyg*; 58: 707–23.
- Basinas I, Sigsgaard T, Heederik D *et al.* (2012b) Exposure to inhalable dust and endotoxin among Danish livestock farmers: results from the SUS cohort study. *J Environ Monit*; 14: 604–14.
- Bernstein IL, Bernstein JA, Miller M *et al.* (1999) Immune responses in farm workers after exposure to *Bacillus thuringiensis* pesticides. *Environ Health Perspect*; 107: 575–82.
- Burdorf A. (2005) Identification of determinants of exposure: consequences for measurement and control strategies. *Occup Environ Med*; 62: 344–50.
- Davies PD, Jacobs R, Mullins J *et al.* (1988) Occupational asthma in tomato growers following an outbreak of the fungus *Verticillium albo-atrum* in the crop. *J Soc Occup Med*; 38: 13–7.
- DECOS. (2010) *Endotoxins. Health-based recommended occupational exposure limit*. The Hague: Health Council of the Netherlands.
- Doekes G, Larsen P, Sigsgaard T *et al.* (2004) IgE sensitization to bacterial and fungal biopesticides in a cohort of Danish greenhouse workers: the BIOGART study. *Am J Ind Med*; 46: 404–7.
- Douwes J, Versloot P, Hollander A *et al.* (1995) Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol*; 61: 1763–9.
- Duquenne P, Marchand G, Duchaine C. (2013) Measurement of endotoxins in bioaerosols at workplace: a critical review of literature and a standardization issue. *Ann Occup Hyg*; 57: 137–72.
- Eduard W. (2009) Fungal spores: a critical review of the toxicological and epidemiological evidence as a basis for occupational exposure limit setting. *Crit Rev Toxicol*; 39: 799–864.
- Eduard W, Heederik D. (1998) Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environments. *Am Ind Hyg Assoc J*; 59: 113–27.
- Farruggia E, Bellia M. (2001) Occupational allergic asthma in greenhouses. Report of a clinical case. *Med Lav*; 92: 203–5.
- Groenewoud GC, de Graaf in 't Veld C, vVan Oorschot-van Nes AJ *et al.* (2002a) Prevalence of sensitization to the predatory mite *Amblyseius cucumeris* as a new occupational allergen in horticulture. *Allergy*; 57: 614–9.
- Groenewoud GC, de Jong NW, Burdorf A *et al.* (2002b) Prevalence of occupational allergy to *Chrysanthemum* pollen in greenhouses in the Netherlands. *Allergy*; 57: 835–40.

- Groenewoud GC, de Jong NW, van Oorschot-van Nes AJ *et al.* (2002c) Prevalence of occupational allergy to bell pepper pollen in greenhouses in the Netherlands. *Clin Exp Allergy*; 32: 434–40.
- Hansen VM, Meyling NV, Winding A *et al.* (2012) Factors affecting vegetable growers' exposure to fungal bioaerosols and airborne dust. *Ann Occup Hyg*; 56: 170–81.
- Hansen VM, Winding A, Madsen AM. (2010a) Exposure to bioaerosols during the growth season of tomatoes in an organic greenhouse using Supresivit (*Trichoderma harzianum*) and Mycostop (*Streptomyces griseoviridis*). *Appl Environ Microbiol*; 76: 5874–81.
- Hansen VM, Eilenberg J, Madsen AM. (2010b) Occupational exposure to airborne *Bacillus thuringiensis kurstaki* HD1 and other bacteria in greenhouses and vegetable fields. *Biocontrol Sci Technol*; 20: 605–19.
- Hanssen VM, Nigatu AW, Zeleke ZK *et al.* (2014) High prevalence of respiratory and dermal symptoms among Ethiopian flower farm workers. *Arch Environ Occup Health*. Epub ahead of print, 15 January 2014.
- Kenny LC, Aitken R, Chalmers C *et al.* (1997) A collaborative European study of personal inhalable aerosol sampler performance. *Ann Occup Hyg*; 41: 135–53.
- Kromhout H, Swuste P, Boleij JS. (1994) Empirical modelling of chemical exposure in the rubber-manufacturing industry. *Ann Occup Hyg*; 38: 3–22.
- Lacey J, Dutkiewicz J. (1994) Bioaerosols and occupational lung disease. *J Aerosol Sci*; 25: 1371–404.
- Latza U, Oldenburg M, Baur X. (2004) Endotoxin exposure and respiratory symptoms in the cotton textile industry. *Arch Environ Health*; 59: 519–25.
- Liebers V, Raulf-Heimsoth M, Brüning T. (2008) Health effects due to endotoxin inhalation (review). *Arch Toxicol*; 82: 203–10.
- Madsen AM. (2006) Airborne endotoxin in different background environments and seasons. *Ann Agric Environ Med*; 13: 81–6.
- Madsen AM. (2011) Occupational exposure to microorganisms used as biocontrol agents in plant production. *Front Biosci (Schol Ed)*; 3: 606–20.
- Madsen AM, Hansen VM, Nielsen SH *et al.* (2009) Exposure to dust and endotoxin of employees in cucumber and tomato nurseries. *Ann Occup Hyg*; 53: 129–38.
- Madsen AM, Tendal K, Frederiksen MW. (2014a) Attempts to reduce exposure to fungi, β -glucan, bacteria, endotoxin and dust in vegetable greenhouses and a packaging unit. *Sci Total Environ*; 468–469: 1112–21.
- Madsen AM, Zervas A, Tendal K *et al.* (2014b) Exposure and preventive measure to reduce high and daily exposure to *Bacillus thuringiensis* in potted plant production. *Ann Occup Hyg*; 58: 664–76.
- Monsó E, Magarolas R, Badorrey I *et al.* (2002) Occupational asthma in greenhouse flower and ornamental plant growers. *Am J Respir Crit Care Med*; 165: 954–60.
- NEG. (2011) Endotoxins. The Nordic Expert Group for criteria documentation of health risks from chemicals and the Dutch Expert Committee on occupational safety. *Arbete och Hälsa*; 2011;45:144. Endotoxins. <http://hdl.handle.net/2077/26596>
- Peretz C, Goren A, Smid T *et al.* (2002) Application of mixed-effects models for exposure assessment. *Ann Occup Hyg*; 46: 69–77.
- Portengen L, Preller L, Tielen M *et al.* (2005) Endotoxin exposure and atopic sensitization in adult pig farmers. *J Allergy Clin Immunol*; 115: 797–802.
- Preller L, Kromhout H, Heederik D *et al.* (1995) Modeling long-term average exposure in occupational exposure-response analysis. *Scand J Work Environ Health*; 21: 504–12.
- Radon K, Danuser B, Iversen M *et al.* (2002) Air contaminants in different European farming environments. *Ann Agric Environ Med*; 9: 41–8.
- Rylander R. (2002) Endotoxin in the environment—exposure and effects. *J Endotoxin Res*; 8: 241–52.
- Rylander R. (2006) Endotoxin and occupational airway disease. *Curr Opin Allergy Clin Immunol*; 6: 62–6.
- Samadi S, van Eerdenburg FJ, Jamshidifard AR *et al.* (2012) The influence of bedding materials on bio-aerosol exposure in dairy barns. *J Expo Sci Environ Epidemiol*; 22: 361–8.
- Schwartz DA, Thorne PS, Jagielo PJ *et al.* (1994) Endotoxin responsiveness and grain dust-induced inflammation in the lower respiratory tract. *Am J Physiol*; 267: L609–L17.
- Simon-Nobbe B, Denk U, Pöll V *et al.* (2008) The spectrum of fungal allergy. *Int Arch Allergy Immunol*; 145: 58–86.
- Skousgaard SG, Thisling T, Bindselev-Jensen C *et al.* (2010) Occupational asthma caused by the predatory beneficial mites *Amblyseius californicus* and *Amblyseius cucumeris*. *Occup Environ Med*; 67: 287.
- Smit LA, Heederik D, Doekes G *et al.* (2008) Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. *Eur Respir J*; 31: 1241–8.
- Smit LA, Spaan S, Heederik D. (2005) Endotoxin exposure and symptoms in wastewater treatment workers. *Am J Ind Med*; 48: 30–9.
- Smit LA, Wouters IM, Hobo MM *et al.* (2006) Agricultural seed dust as a potential cause of organic dust toxic syndrome. *Occup Environ Med*; 63: 59–67.
- Spaan S, Wouters IM, Oosting I *et al.* (2006) Exposure to inhalable dust and endotoxins in agricultural industries. *J Environ Monit*; 8: 63–72.
- Thilising T, Sherson D, Madsen AM *et al.* (2012) Type 1 sensitization against a *Steinernema feltiae* product. *BMJ Case Rep*; 2012: 1–3.
- Wang XR, Eisen EA, Zhang HX *et al.* (2003) Respiratory symptoms and cotton dust exposure; results of a 15 year follow up observation. *Occup Environ Med*; 60: 935–41.
- Zuskin E, Schachter EN, Mustajbegovic J. (1993) Respiratory function in greenhouse workers. *Int Arch Occup Environ Health*; 64: 521–6.